

## WHAT IS CLAIMED:

1. A fusion protein comprising a heterologous polypeptide fused to a major coat protein of a virus, wherein the major coat protein is a variant of a wild type major coat protein of the virus.
2. The fusion protein of claim 1, wherein the virus is selected from the group consisting of a filamentous phage, a lambda phage, a Baculovirus, a T4 phage and a T7 phage.
3. The fusion protein of claim 1, wherein the phage is a filamentous phage, the major coat protein is gpVIII and the heterologous polypeptide is fused to the N-terminus or the C-terminus thereof.
4. The fusion protein of claim 1, wherein the major coat protein is a filamentous phage coat protein variant which contains at least one amino acid residue selected from the list below in the position indicated:

	<u>Residue Number</u>	<u>Amino Acid Residue</u>
	1	E, L, V, Q, D, I, N
	2	R, H, F, W, E, K, Y, D
20	3	T, E, L, S, D, I, V, A
	4	D, R, H, E, K
	5	R, H, N, D, K, Q, E
	6	Y, W, S, I, L, F, T, V
	7	T, N, S
25	8	D, H, R, E, K
	9	E, Q, T, D, N, S
	11	W, I, V, Y, L, F
	12	R, H, N, E, D, K, Q
	13	I, L, E, Q, A, V, D, T, N, S
30	14	L, I, V
	15	D, R, N, E, K, H, Q
	16	E, V, L, T, D, I, A, S, G
	17	E, V, L, I, A, T, D
	18	L, V, I
35	19	L, T, Q, E, I, V, S, A, N, D
	20	R, D, H, N, Q, K, E
	21	W, Y, I, L, F, V
	22	W, F, Y

	23	W, Y, I, V, H, K, F, L, R
	24	I, Q, L, N, V
	25	S, L, I, T, V
	26	A, I, V, G, L, M
5	27	N, T, S
	28	I, L, V
	29	K, R, F, W, H, Y
	30	I, V, L.

- 10 5. A fusion protein comprising a heterologous polypeptide fused to at least a portion of a coat protein of a filamentous phage, wherein the coat protein is a variant of a wild type coat protein of the phage, the variant having an alteration in the transmembrane domain or in the cytoplasmic domain of the coat protein.
- 15 6. The fusion protein of claim 5, wherein the coat protein is gpIII of a filamentous phage and the heterologous polypeptide is fused to the N-terminus or the C-terminus thereof.
7. The fusion protein of claim 1, wherein the variant has 2 - 50 altered residues relative to the wild type coat protein sequence.
- 20 8. The fusion protein of claim 1, wherein the heterologous polypeptide is an antibody or a fragment thereof or a cytokine or a cytokine receptor.
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9. A replicable expression vector comprising a gene fusion, wherein the gene fusion encodes the fusion protein of claim 1.
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10. A library comprising a plurality of the replicable expression vectors of claim 9, the expression vectors comprising a plurality of different gene fusions encoding a plurality of fusion proteins.
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11. Host cells comprising the vector of claim 20.
12. A virus displaying the fusion protein of claim 1 on the surface thereof.
- 35 13. A library of virus, comprising a plurality of the virus of claim 12 displaying a plurality of different fusion proteins on the surface thereof.
14. A method, comprising:

constructing a library of phage or phagemid particles displaying a plurality of the fusion protein of claim 1;

contacting the phage or phagemid particles with a target molecule so that at least a portion of the particles bind to the target molecule; and

5 separating the particles that bind from those that do not bind.

15. A method of decreasing the detection limit of a phage display system utilizing a phage containing a gene fusion encoding a fusion protein, where the gene fusion comprises a first gene encoding a heterologous polypeptide and a second gene encoding at least a portion of a phage coat protein, the method comprising mutating the second gene to encode a variant of a wild type coat protein of the phage. }

16. A method of transforming cells, comprising electroporating cells in the presence of heterologous DNA under conditions suitable to allow the heterologous DNA to enter the cells, 15 wherein the heterologous DNA is purified by affinity purification.

17. The method of claim 16, wherein the heterologous DNA is present at a concentration of about 1 picogram to about 500 micrograms/mL.

20 18. The method of claim 16, obtaining at least  $1 \times 10^{10}$  transformants in one electroporating step.

19. The method of claim 16, wherein the cells are present at a concentration of about  $1 \times 10^{11}$  to about  $4 \times 10^{11}$  cfu/mL.

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20. The method of claim 19, wherein the cells are *F'::Tn10 proA<sup>+</sup>B<sup>+</sup>lacI<sup>q</sup>D(lacZ)M15/ F-araD139D(ara-leu)7696galE15galK16D(lac)X74rpsL(Str<sup>r</sup>)hsdR2(rk<sup>-</sup>mk<sup>+</sup>)mcrAmcrB1 E. coli* cells.

30 21. A method for producing a product polypeptide, comprising the steps of:  
(1) culturing a host cell transformed with a replicable expression vector, the replicable expression vector comprising DNA encoding a product polypeptide operably linked to a control sequence capable of effecting expression of the product polypeptide in the host cell;

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wherein the DNA encoding the product polypeptide has been obtained by a method comprising the steps of:

(a) constructing a family of variant replicable plasmids comprising a transcription regulatory element operably linked to a gene fusion encoding a fusion protein, wherein the gene fusion comprises a first gene encoding a polypeptide and a second gene encoding at least a portion of a phage coat protein, wherein the variant replicable plasmids comprise variant first genes encoding variant polypeptides;

(b) transforming suitable host cells with the plasmids using the method of claim 16;

(c) optionally, when the plasmid is a phagemid which requires a helper phage to produce phage particles, infecting the transformed host cells with an amount of helper phage encoding the phage coat protein sufficient to produce recombinant phagemid particles, preferably wherein no more than a minor amount of the phagemid particles display one or more copies of the fusion protein on the surface of the phagemid particles;

(d) culturing the transformed infected host cells under conditions suitable for forming recombinant phage particles containing at least a portion of the plasmid and capable of transforming the host cells;

(e) contacting the recombinant phage particles with a target molecule so that at least a portion of the phage particles bind to the target molecule;

(f) separating phage particles that bind to the target molecule from those that do not bind;

(g) selecting one of the variant polypeptides encoded by the plasmid in a phage particle which binds or does not bind to the target molecule as the product polypeptide and cloning DNA encoding the product polypeptide into the replicable expression vector; and

(2) recovering the expressed product polypeptide.

22. A fusion protein comprising at least a portion of a protein III or protein VIII filamentous phage coat protein having a heterologous polypeptide fused to the carboxyl-terminus thereof.

23. A replicable expression vector comprising a gene fusion, wherein the gene fusion encodes the fusion protein of claim 22.

24. A library comprising a plurality of the replicable expression vectors of claim 23, the expression vectors comprising a plurality of different gene fusions encoding a plurality of fusion proteins.

25. Host cells comprising the vector of claim 23.

26. A virus displaying the fusion protein of claim 22 on the surface thereof.

27. A library of virus, comprising a plurality of the virus of claim 26 displaying a plurality of different fusion proteins on the surface thereof.
- 5 28. A method, comprising:  
constructing a library of phage or phagemid particles displaying a plurality of the fusion protein of claim 22 on the surface thereof;  
contacting the phage or phagemid particles with a target molecule so that at least a portion of the particles bind to the target molecule; and  
10 separating the particles that bind from those that do not bind.